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A BUFFERED PHYSIOLOGIC SALT SOLUTION

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The importance of the relationship between H-ion concentration of the medium and biologic phenomena has received much attention in recent years. But the results of the researches of Sørensen, Michaelis, Henderson, Clark and others have not yet been practically applied to every phase of biologic investigation. In the field of immunology the various tests are generally carried on without the degree of consideration for control of H-ion concentration which it is practically possible to attain. It is the purpose of this paper to present a simple method for the control of the H-ion concentration of physiologic salt solution.

Even when all due care is taken for the cleanliness of receptacles, the range of variation in reaction of unbuffered solutions is considerable. Distilled water standing in hard glass flasks, protected from the atmosphere only by a cotton plug, has been found to absorb enough carbon dioxide to bring the reaction to P_H 5.8. On the other hand, distilled water which had been sterilized and left standing in soft glass cylinders with ground glass stoppers was found with a reaction of P_H 8.8. These figures are merely variations which have come under the writer's observation. Recently Esty and Cathcart [1] have presented data showing the variations in H-ion concentration of unbuffered solutions sterilized in hard and soft glass. They tested the variations in 0.85% sodium chloride solution, distilled water, and in weak solutions of hydrochloric acid and sodium hydroxide. Their results show an increase in alkalinity as the heating is prolonged in soft glass tubes, whereas an increase in acidity was observed in hard glass tubes.

The influence of variation in H-ion concentration of the medium has not been determined for certain serologic reactions used commonly for test purposes. But there is a general recognition of a relationship between H-ion concentration and agglutination. For theoretical considerations the reader is referred to a recent paper by Coulter.[2] A single illustration of the influence of H-ion concentration on agglutination is given in table 1.

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[1] J. Infect. Dis., 1921, 29, p. 29.

[2] J. Gen'l Phys., 1921, 3, p. 309.

The tests for which the data are given in table 1 were made with monovalent meningococcus rabbit serum. The immunizing strain and test strain belonged to type 2. The antigen was killed by heat and made up to a standard density so that the final suspension contained the equivalent of 500 part per million of silica standard. Antigen suspensions and serum dilutions were made up in salt solutions varying in H-ion concentration. They were held at the desired P_H values by the use of buffer mixtures, as described further on. For the tests the various antigen suspensions were added to the corresponding serum dilutions. Incubation took place over night in a water bath at 56 C.

TABLE 1

INFLUENCE OF H-ION CONCENTRATION ON AGGLUTINATION OF MENINGOCOCCI

H ion Concentration	Salt Solution Alone	Normal Serum 1:50	Immune Serum Diluted 1 Part in:					
			50	100	200	400	800	1600
p_H 5.6	0	0	4	4	4	4	4	3
p_H 6.0	0	0	4	4	4	4	4	3
p_H 6.4	0	0	4	4	4	4	3	2
p_H 6.8	0	0	4	4	4	4	3	2
p_H 7.0	0	0	4	4	4	3	3	1
p_H 7.4	0	0	4	4	4	3	2	1
p_H 7.7	0	0	3	4	4	3	2	1
p_H 8.0	0	0	3	4	4	3	2	1
p_H 8.6	0	0	4	4	4	3	2	1

4 = complete agglutination; 3 = turbidity in a control tube containing 75% as much antigen as in the tubes in which the test was carried out; 2 = turbidity in a control tube containing 50% of the antigen; 1 = turbidity in a control tube containing 25% of the anitigen.

According to the method used for reading the results of routine tests at the Hygienic Laboratory, by which reactions expressed by 4 and 3 are considered positive, and those expressed by 2 and 1 are considered negative, this serum had a titer of 1:400 when the reaction was P_H 7.4; a titer of 1:800 when the reaction was P_H 7; and a titer of 1:1,600 when the reaction was P_H 6.

Russell, Nichols and Stimmel[3] found that there was a wide variation in the reaction of typhoid vaccines, and they devised a method by which variations in either direction could be controlled. A solution of 8.7 gm. KH_2PO_4 and 165 gm. K_2HPO_4 in one liter of distilled water was prepared. Two c c of this solution added to each liter of 0.85% NaCl solution used in making the vaccine was found to hold the reaction at about P_H 7.4. The reaction of the buffer solution was about P_H 8, that of the buffered salt solution P_H 7.4.

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[3] Milit. Surgeon, 1920, 47, p. 359.

The simple formula here proposed for a buffered salt solution would be applicable to the preparation of a salt solution with a controlled H-ion concentration for any biologic use except when it is desirable to have calcium in the solution. (The calcium would combine with the phosphates to form an insoluble precipitate.)

The controlling agent in the buffered salt solution is a phosphate mixture prepared according to Sørenson. The $\frac{m}{15}$ primary (KH_2PO_4) and secondary ($Na_2HPO_4 . 2 H_2O$) phosphates are mixed together in the proportions given in Sørensen's tables to obtain any desired P_H value between 5.3 and 8.

In practice the procedure is as follows: A $\frac{m}{15}$ solution of primary phosphate is prepared by adding 9.078 gm. KH_2PO_4 to 1 liter of distilled water. A $\frac{m}{15}$ solution of secondary phosphate is prepared by adding 11.876 gm. $Na_2HPO_4 . 2 H_2O$ to 1 liter of distilled water. A mixture of 6 c c of the secondary solution and 4 c c of the primary solution has a P_H value very near 7. A mixture of 8 c c of the secondary solution and 2 c c of the primary solution has a P_H value very near 7.4. A solution of any other desired concentration within the range of the phosphate mixture can be obtained by altering the proportions of primary and secondary solutions.

In ordinary routine work commercial chemicals and a rough balance can be used. The mixtures should be controlled by comparing with standard buffer solutions, adjusting to the desired H-ion concentration by adding a little more of the acid or alkaline solution as required. It should be borne in mind that the commercial secondary phosphate contains 12 mols of water of crystallization, and if used in the buffer mixtures the $\frac{m}{15}$ solution should be calculated accordingly. The salt containing 2 mols of water of crystallization is prepared by exposing to the ordinary atmosphere the crystals containing the 12 mols of water. About 2 weeks' exposure is sufficient. For further information regarding Sørensen's standard buffer solutions the reader is referred to Clark, "The Determination of Hydrogen Ions," from which this information was obtained.

For the preparation of the isotonic buffered salt solution 1 part of phosphate mixture of the desired H-ion concentration is added to 9 parts of 0.9% NaCl solution.

The efficacy of the buffered salt solution to maintain the desired H-ion concentration, by taking care of the variations in reaction to which laboratory solutions treated with due care are subject, was tested. Unbuffered salt solution was rendered acid or alkaline by the addition of HCl or NaOh. To 9 parts of these salt solutions 1 part of phosphate mixture P_H 7 was added. The salt solutions with H-ion concentrations up to P_H 3.8 were completely neutralized by the buffer. Those with P_H values of 3.4 and 3.6 had a P_H value of 6.9 and the P_H 3 solution had a P_H value of 6.8 after the addition of the buffer. The alkaline solutions with P_H values up to 10 were completely neutralized by the buffer. But the buffer was not capable of controlling perfectly the alkali in a sample of water, said to be distilled, which had been sterilized in a soft glass container. The water had a P_H value of 8.8. When the buffer was added the solution had a H-ion concentration of 7.2. This illustrates how the efficiency of the buffer depends on the nature of the solution to which it is added.

The P_H values recorded in this paper were determined by the use of the colorimetric method. There are certain errors which would be detected by the electrometric method, but these are negligible from a practical point of view when buffered solutions are used in serologic tests.